Vasoactive Substances in Blood and Urine of DCA Hypertensive Rats

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H. Croxatto et al. have demonstrated that on incubation for 24 hours at 38°C. and pH 3.8 to 4, the serum of each of the animals they studied (including mammals, birds, and amphibia) produced a peptide which causes smooth muscle contraction. This peptide has been named "anephrotensin." Slight pharmacological differences were found among the anephrotensins obtained from the several types of animals, but each was capable of raising the blood pressure of the nephrectomized animal into which it was injected intravenously. Rat anephrotensin, injected intravenously, lowered the blood pressure of normal rats; added to the bath solution it caused the contraction of isolated smooth muscle from the ileum of the guinea pig, the caecum rectalis of the chicken, and the uterus of the rat. The last named is the most sensitive of the tissues studied. A further finding from their studies is that serum from nephrectomized animals yields much greater anephrotensin activity than comparable serum from normal animals.

Differences between normal and nephrectomized animals in their response to, and production of, anephrotensin led us to inquire whether there are similar differences in these respects between normal rats and rats made hypertensive by DCA treatment; and, further, to see whether there are any detectable changes in kidney function which might be associated with differences in the anephrotensin recovered from the serum.

Methods

1. PREPARATION OF EXPERIMENTAL ANIMALS

Fifty-one adult, male, uninephrectomized rats each received a daily subcutaneous injection consisting of 1 mg. of microcrystals of DCA, suspended in 0.2 ml. of distilled water. Forty-six similarly uninephrectomized animals, which did not receive DCA, served as controls. Both groups were given a mixture of 0.75 per cent NaCl and 0.25 per cent KCl as drinking solution. Blood pressure measurements were made at weekly intervals and at the time of the acute experiment, using the tail plethysmograph method described by Sobin. The last are the values used in figure 1. After 10 weeks, the blood pressure of the DCA-treated animals was definitely higher than that of the controls. This was increasingly so with longer periods of treatment, up to the 30 weeks of the experimental period.

2. PREPARATION OF STOCK ANEPHROTENSIN

Blood was collected from the abdominal aortae of rats anesthetized with bromethol, 250 mg./Kg. body weight intraperitoneally (Avertin, Winthrop), and centrifuged at 3 to 4°C. The serum was acidified (pH 4) with HCl and incubated under toluene at 35°C. for 20 to 24 hours, after which it was precipitated with 9 volumes of 95 per cent ethanol. The supernatant, containing the active component, was evaporated in vacuo at a temperature below 40°C. The active material was subjected to further purification by being redissolved in 5 to 10 ml. of glacial acetic acid and reprecipitated with 10 volumes of ether. This precipitate was dissolved in saline (one-half the volume of the original serum) for injection into DCA-treated and control animals.

3. ACTION OF ANEPHROTENSIN ON THE BLOOD PRESSURE OF CONTROL AND DCA-TREATED RATS

Thirty-six rats treated with DCA for periods of 2 to 29 weeks, and thirty-two control rats, were used in this study. Animals were anesthetized (1 ml./Kg. body weight I.M.) with a solution containing 10 Gm. diallylbarbituric acid, 40 Gm. urethan, 40 Gm. monethylurea, and distilled water up to 100 ml. Blood pressure was recorded from the left common carotid artery by means of a
membrane manometer, which had been calibrated with a mercury manometer. After the blood pressure level was established, the animal received a 0.1-nil. injection containing the anephrotensin obtained from 0.2 ml. of normal rat serum. A value reflecting both magnitude and duration of response was obtained from the blood pressure recordings by measuring the area of the tracings above and below a line representing the basal pressure (see fig. 2).

4. COMPARISON OF THE ANEPHROTENSIN YIELD OF SERUM FROM NORMAL AND DCA-TREATED RATS

In these experiments, blood from which anephrotensin was to be prepared was taken from the aortae of rats, control and DCA-treated, in which the circulation between head and trunk had been stopped abruptly by a tourniquet placed around the neck. This was done in order to avoid the passage of vasopressin and oxytocin produced during acute hemorrhage. The subsequent treatment of the serum for anephrotensin production was as described in paragraph 2 above. Activity of serum extracts from 20 pairs of treated and control rats was assayed using isolated rat uterine tissue suspended in a modified Tyrode's solution to which 1 mg. atropine and 10 mg. dibenamine per liter was added, in order to block acetylcholine and adrenergic substances. Synthetic oxytocin (Syntocinon, Sandoz) was used as a standard.

5. DETERMINATION OF ANEPHROTENSIN-LIKE SUBSTANCES IN THE URINE OF NORMAL AND DCA-TREATED RATS

Urine samples were collected, under toluene and glacial acetic acid, at weekly intervals (2 to 16 weeks), from groups of 4 to 8 DCA-treated rats, and similarly from groups of control rats. Uterotonic components were separated out by the method of Noble et al. and were assayed with rat uterine tissue as described above. The effect of this material on blood pressure of normal and nephrectomized rats was determined in eight cases.

6. DETERMINATION OF PRESSOR SUBSTANCES IN KIDNEY OF NORMAL AND DCA-TREATED RATS

Homogenized extracts were prepared from kidney of rats after periods of 4 to 30 weeks of DCA treatment. Similar extracts were prepared from kidneys of control rats. The method of preparation was as follows: Excised kidneys were ground in a mortar with coarse sand; saline was added, 5 ml./Gm. of tissue; the mixture was centrifuged and the supernatant so diluted that the extract

*The composition of the Tyrode's solution in mEq/L. was: NaCl, 137; KCl, 2.7; CaCl₂, 0.4; MgCl₂, 2.0; NaHCO₃, 12.0; Na H₂PO₄, 0.36; dextrose, 5.6.

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Blood pressure responses of 32 control rats and 36 rats treated with DCA for periods of 2 to 30 weeks to anephrotensin obtained from 0.2 ml. normal rat serum.

from 10 mg. of tissue was contained in 1 ml. of solution. This extract was assayed by determining the effect of 0.1 ml. I.V. on the blood pressure of a rat previously nephrectomized (16 hours). Evaluation of the results was made as in paragraph 3.

Results

ACTION OF ANEPHROTENSIN ON BLOOD PRESSURE OF DCA-TREATED RATS

After five weeks of DCA treatment, some rats showed a blood pressure elevation in response to anephrotensin injection (fig. 3). The percentage of rats responding so, and the magnitude of the elevation, increased from the fifth to the tenth week, after which both remained fairly constant for the duration of the experiment (30 weeks). Blood pressure of untreated rats and of most rats treated for less than five weeks was lowered by anephrotensin; blood pressure of all hypertensive treated animals and of 8 of 14 treated but not hypertensive animals (b.p. < 140 mm. Hg) was increased by anephrotensin injection.

INFLUENCE OF DCA ON THE ANEPHROTENSIN-PRODUCING CAPACITY OF RAT SERUM

Serum extract obtained from rats treated with DCA for eight weeks or more had greater uterotonic activity, after incubation at pH 4, than did that from control rats, and though there was considerable variation, the activity was greater as the treatment period was longer (fig. 4). Serum extract from rats treated less than eight weeks in general had less uterotonic activity than that from control rats.

INFLUENCE OF DCA TREATMENT ON THE ANEPHROTENSIN-LIKE SUBSTANCES EXCRETED BY THE KIDNEY

The urine of rats which had undergone DCA treatment for 2 to 16 weeks was found to have greater uterotonic activity than that of control rats and, in general, the activity was greater as the period of treatment was longer (fig. 5). The average oxytocic activity per 100 Gm. body weight in 10 hours was equivalent to $2.71 \pm 0.87$ mU. oxytocin in the control group, and to $13.28 \pm 3.02$ mU. in the DCA-treated group. This difference has a $P$ value of < 0.0005.

These urine extracts from control and DCA-treated rats were predominantly hypotensive in their effect on the blood pressure of normal rats and predominantly hypertensive in the nephrectomized rats.

INFLUENCE OF DCA TREATMENT ON THE PRESSOR ACTION OF HOMOGENIZED RAT KIDNEY

Kidney extract from DCA-treated rats had less pressor activity than that from control rats.
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rats, and the activity decreased rapidly during the first five weeks of the treatment period (fig. 6). Kidney extracts of rats treated 10 weeks or more showed practically no pressor activity.

Discussion

The data reported here show that the blood pressure of DCA-treated rats is elevated by intravenous injection of anephrotensin, whereas that of uninephrectomized control rats is depressed. Furthermore, they show that there is significantly more anephrotensin activity in serum extract of treated rats than in that of the controls. This suggests that DCA treatment induces a humoral change that favors the formation of anephrotensin.

It is possible that anephrotensin, or some similar peptide, may play a role in corticoid hypertension. The presence of such a substance in the internal environment of the hypertensive rats has not been demonstrated; however, early increase of the urinary excretion of uterotonic substances similar to anephrotensin in their action on the blood pressure of normal and nephrectomized rats may indicate the presence of a similar peptide in the blood of DCA-treated rats.

Anephrotensin has not been isolated, but its pharmacological properties suggest that it is different from other polypeptides of known chemical structure, such as vasopressin, oxytocin, angiotensin, and bradykinin. It also differs from the vasopressor substance obtained from incubated plasma by Khairallah and Page, which is a lipid.

Our data confirm previous reports that in rats submitted to prolonged DCA treatment there is a significant decrease of the renin-like substance in the kidney. These results argue against an overactivity of the renin-angiotensin mechanism, as do results of experiments with other forms of chronic experimental hypertension. The bulk of evidence favors the concept that the onset of DCA hypertension coincides with a striking decrease of renin in the kidney.

There are similarities between corticoid-hypertensive rats and nephrectomized rats in addition to that of the disappearance of renin, e.g., higher sensitivity to anephrotensin and higher serum yield of this polypeptide. Apparently what nephrectomy produces in a few hours appears slowly in DCA-treated rats. The similarity between nephrectomized rats and those treated with high DCA doses strengthens the concept that the humoral alterations and changes in the sensitivity of the blood vessels come into play when some protective function of the kidney is missing.
The observations are compatible with the possibility that the normal kidney maintains normotension by eliminating the effect of an extrarenal pressor agent such as anephrotenisin.

Summary

The effect of intravenous injection of rat anephrotenisin on rats treated with DCA for periods of up to 30 weeks was studied. It was found that in these animals, anephrotenisin raises blood pressure, whereas in controls it acts as a depressor. Blood serum of DCA-treated rats yielded greater anephrotenisin activity after acid incubation than did that of controls. Treated animals excreted, through the kidneys, more anephrotenisin-like substance than controls. The pressor effect of the homogenized kidneys of these animals was less than that of controls; this is attributed to decrease in renin. Higher anephrotenisin yield and the pressor response to anephrotenisin were found to occur previous to the onset of hypertension; this suggests a causal relationship with the hypertension-provoking mechanism. The observations point to a similarity between DCA-treated and nephrectomized rats.

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